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Research Article

Exploring the Potential Applications of *Withania Somnifera*

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Abstract

Withania somnifera commonly known as Ashwagandha, a medicinal ayurvedic herb belongs to the family Solanaceae. In our study, the medicinal properties of aqueous root extract of *Withania somnifera* were investigated. The phytochemical tests confirmed the presence of flavonoids and absence of alkaloids, saponins and tannins. The antioxidant activity increased in a dose dependent manner. In anticancer activity, the highest percentage of inhibition was observed at a concentration of 500µg/ml in MCF-7 cells. The antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Aspergillus niger* was checked and observed potential inhibition against *Pseudomonas aeruginosa*. Biochemical assessment of anti-inflammatory and anti-diabetic activities was performed, where *W. somnifera* showed maximum percentage of inhibition at 1000µg/ml and a reasonable percentage of inhibition at 200µg/ml respectively. *In-vitro* glucose uptake activity was carried out in 3T3-L1 cell line, followed by expression of Glut-4 gene. The highest wound closure rate was observed at 200µg/ml. Thus we conclude that aqueous root extract of *W. somnifera* showed numerous therapeutic potential which makes it a valuable natural source.

Keywords: *Withania somnifera, Phytochemical, Antioxidant, Anti-cancer, Antimicrobial, Anti-inflammatory, Anti-diabetic, Glucose uptake, Glut-4, 3T3-L1, Wound healing*.

Introduction

W. somnifera (ashwagandha/winter cherry) is one of the most accepted and studied medicinal herb due to its availability all around the world (Scartezzini and Speroni, 2000). It is an important ancient plant in Indian traditional system of medicines, Ayurveda and Unani (Umadevi, *et al*., 2012). Plants produce non-nutritive elements called phytochemicals, which are responsible for their medicinal properties. Phytochemical (withanolides) present in *W. somnifera* has many pharmaceutical properties such as anti-oxidant, anti-inflammatory, anti-diabetics, anti-cancer and anti-microbial (Khanchandani, *et al*., 2019). Antioxidant property plays a crucial role in protecting skin from free-radicals. Withaferin A (glycowithanolides) present in *W. somnifera* extract plays a significant role as free-radical scavengers (Bhattacharya, *et al*., 2001). Studies have demonstrated that aqueous extract of *W. somnifera* is a powerful antioxidant agent which can prevent cancer cell growth (Wafaa, *et al*., 2018) & amp; (Panjamurthy, *et al*., 2008). The chemical study using the parts of *W. somnifera* has shown that it can treat both tumour and inflammation due to the presence of bioactive compounds called withanoids (Girdhar and Rana, 2007). Due to the presence of Withaferin A, the growth of many gram positive, pathogenic fungi and aerobic bacilli has inhibited. *W. somnifera* showed inhibiting activity towards *Pseudomonas aeruginosa (P. aeruginosa)* strains (Barnes, *et al*., 2015). The inflammatory response is a natural defence system which includes complex outpouring steps, due to the activities of white blood cells and its other components (Jinu, 2014). The oral administration of root powder of *W. somnifera* significantly lowered blood glucose in diabetic patients (Andallu and Radhika, 2000). Furthermore, *W. somnifera's* ability to act as an insulin secretagogue is quite remarkable (Jonathan, *et al*., 2015). The most significant events in glucose homeostasis is insulin stimulated glucose transport by glucose transporters and among the glucose transporters, GLUT- 4 plays a major role in transporting glucose to muscles (Joost, *et al*., 2001). Wound healing activity was estimated using a blend of herbal

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drugs which includes *Rubia cordifolia, Centella asiatica*, *Terminalia belerica*, *Plumbago zeylanica* and *Withania somnifera* on ablated wound of albino rats. It was detected that the herbal blend of drugs promotes wound healing in animals (Gupta, *et al*., 2011).

In this study, the phytochemical, antioxidant, anticancer, antimicrobial, anti-inflammatory, anti-diabetic, glucose uptake and wound healing properties of *W. somnifera* were analysed.

Materials and methods

Collection of *W. somnifera*

Root powder of *W. somnifera* was collected from the local area of Thiruvananthapuram.

Preparation of crude extract of *W. somnifera*

5 grams (g) of root powder was weighed and mixed with 200 millilitre (ml) of distilled water (DW). The sample was then filtered using muslin cloth. Then the extract was continuously stirred using a magnetic stirrer for 6 hours (hrs). After 6 hrs the sample was taken and kept in a boiling water bath for 24hrs at 90 Degree Celsius (°C) until fully dried without any moisture content. The dried sample was then weighed and used for further experiments.

Phytochemical analysis of *W. somnifera*

The prepared crude extract of *W. somnifera* were analysed and screened for the identification of bioactive chemical compounds.

Test for Tannins

Few drops of ferric chloride were mixed with aqueous solution of *W. somnifera.* Blue colour indicated the presence of tannins.

Test for Alkaloids

Few drops of wagner's reagent were added into the aqueous solution of *W. somnifera*. Reddish-brown colour indicated the presence of alkaloids.

Test for Flavonoids

To the aqueous solution of *W. somnifera* a few drops of sodium hydroxide was added followed by diluted hydrochloric acid (HCL). Vanishing of yellow colour on adding diluted acid indicated the presence of flavonoids.

Test for Saponins

Aqueous solution of *W. somnifera* was mixed vigorously. Presence of consistent foam indicated the presence of saponins.

Antioxidant assay of *W. somnifera*

Reducing power activity (RPA) is the modest method to determine the antioxidant activity of *W. somnifera*. Various concentrations of aqueous solution of *W. somnifera* (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml) were taken and mixed with 2.5ml of 0.2 molar (M) sodium phosphate buffer (Na-PB) (pH 6.6) followed by 2.5ml of freshly prepared 1% potassium ferric cyanide ($K_3Fe(CN)_6$ solution. The solution was then mixed well and kept inside the water bath at 50°C for 20 minutes (mins). After 20 mins, 2.5ml of 10% trichloroacetic acid (TCA) was added to the mixture and shaken well. The mixture was then centrifuged at 4000 rpm for 10mins, and the supernatant was mixed with DW followed by freshly prepared 0.5ml of ferric chloride ($FeCl_3$) solution (0.1%). Incubated at room temperature (RT) for 10 mins. The absorbance was measured at 700nm using spectrophotometer. The higher the absorbance value indicates the higher reducing power ability. Colour of the solution changed from colourless to blue.

Anti - cancer activity

Anticancer activity has been studied using different concentrations of aqueous root extract of *W. somnifera* with different concentrations (50µg/ml, 200µg/ml, 300µg/ml & 500µg/ml) on MCF-7 (human breast adenocarcinoma cell line) showed high proliferating capacity. MCF-7 cells were seeded in 24-well plates. The cells were cultured in media containing DMEM (Dulbecco's Modified Eagle Medium), FBS (Foetus Bovine Serum), Antibiotics and incubated in CO_2 incubator at 37°C for 48hrs. After 48hrs, aqueous root extract of *W. somnifera* was added and incubated for 24hrs. After 24hrs, the media was discarded followed by PBS wash and addition of MTT (3- (4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) and again incubated for 3 - 4hrs at 37°C. The cells were observed under inverted microscope for the

presence of formazan crystals, later the formazan crystals were diluted using DMSO (Dimethyl Sulfoxide) and kept for further incubation, 15 mins at RT. Absorbance was taken at 570nm using spectrophotometer

Cell Viability and Percentage of Inhibition can be calculated using the formula: -

Cell viability % = (Test OD/Control OD) X 100
Percentage of inhibition = (Control OD – Test OD/Control OD) X 100

Antimicrobial activity

Agar well diffusion method has been used to study the antimicrobial activity of aqueous root extract of *W. somnifera* at different concentrations (50µg/ml and 200µg/ml) against *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Aspergillus niger* (*A. niger*). Nutrient media was prepared using NB and agar, PDA media was used for *A. niger*. The media was then poured into sterilized petri plates and allowed to solidify. After the solidification, the inoculum was uniformly spread using a sterile cotton swab on the sterile petri dish agar media. Wells were then punched out. Gentamicin was taken as PC and DW as negative control (NC). Samples were then incubated for 24 hrs at 37°C. After the incubation, the size (diameter) and zone of inhibition was observed and measured.

Anti-inflammatory activity

Anti-inflammatory activity has been studied using different concentrations of aqueous root extract of *W. somnifera* (800µg/ml, 1000µg/ml, 1200µg/ml). The samples were taken and mixed with 1000µl of bovine serum albumin (BSA) followed by 1000µl of phosphate buffered saline (PBS). Aqueous root extract of *Syzygium cumini* (*S. cumini*) was taken as PC and DW was taken as Control. The samples were mixed well and kept for incubation at RT (37°C) for 5 mins. After the incubation at RT, the samples were kept in water bath for 5 mins at 70°C. The absorbance was measured at 660nm using spectrophotometer. The % inhibition of protein denaturation was calculated using the formula:

$$\% \text{ inhibition} = (1 - [A_2 / A_1] \times 100)$$

Where;
A_2 = Absorbance of Test sample
A_1 = Absorbance of Control

Anti-diabetic activity

Anti-diabetic activity has been studied using different concentrations of aqueous root extract of *W. somnifera* (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml). The samples were taken and mixed with 100µl of Na-PB (pH 6.6) followed by 100µl of α-amylase. The solution was then mixed well and kept for incubation at 30°C for 10mins. After 10mins, 1000µl of starch is added, mixed well and kept for incubation at 30°C for 10 mins. After 10 mins, 1000µl of dinitro salicylic acid (DNS) solution were added and mixed well and kept for an incubation period of 5 mins at 30°C. After the incubation period, the samples were allowed to cool followed by the addition of 100µl of DW. Acarbose was taken as positive control. Absorbance was measured at 540nm using spectrophotometer. Colour of the solution changed from colourless to cloudy white.

% Inhibition can be calculated using the formula:

$$\% \text{ of Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Glucose uptake activity

Glucose uptake activity was studied using aqueous root extract of *W. somnifera* (at a concentration of 200µg/ml), 3T3-L1 cells were seeded in 6-well plate. The cells were cultured in media containing DMEM, FBS, antibiotics and incubated in CO_2 incubator at 37°C for 48hrs. After 48hrs the adherent cells were starved under serum free media (only DMEM) and incubated for 16hrs in CO_2 incubator at 37°C. After 16hrs of starvation, serum free media was discarded. Then about

1ml of glucose (0.01mg/ml) was added to each well. 1 ml of glucose along with 0.5ml of insulin was considered as PC. 500µl was immediately taken for initial estimation of glucose using anthrone method. Then the cells were incubated for 30 mins. After incubation about 500µl of the solution was taken for final glucose estimation using anthrone method. Absorbance was taken at 620nm using spectrophotometer.

Concentration of glucose uptake was calculated from the standard curve of glucose (absorbance at 620nm on Y-axis and different concentration of standard glucose on X-axis).

% of Glucose uptake can be calculated using equation: -

Absorbance of Final / Absorbance of Initial x 100

Expression of Glut- 4 on 3T3-L1 cell line

3T3-L1 cells were cultured and DNA was isolated, precipitated in TE buffer, its quality was assessed using agarose gel electrophoresis. To evaluate GLUT-4 expression, PCR was executed on cells treated with glucose and a sample containing aqueous root extract of *W. somnifera* (concentration 200µg/ml) while untreated cells served as the control.

Glut-4 F - 5′-GTAACTTCATTGTCGGCATGG-3′

Glut-4 R - 5′- AGCTGAGATCTGGTCAAACG- 3′

The amplification reaction was performed in a thermal cycler using the PCR conditions with the steps of initial denaturation at 94°C for 10 mins followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 2 mins. The final extension was done at 72°C for 7 mins and cooling at 4°C for ∞ seconds.

The PCR reaction products were electrophoresed in 1.5% agarose gel and visualized using UV transilluminator to confirm the gene expression.

Wound healing activity

Wound healing activity has been studied using different concentrations of aqueous root extract of *W. somnifera* (50µg/ml, 100µg/ml, 150µg/ml, and 200µg/ml) on L929 fibroblast cell line derived from connective tissue of male mouse, and are suitable for studying cell migration and wound healing. L929 cells were seeded in 6-well plate. The cells were cultured in media containing DMEM, FBS and antibiotics were pre-incubated in CO_2 incubator at 37°C for 48hrs. After 48hrs, the plates were taken and using a 200µl sterile tip a scratch was introduced followed by the addition of aqueous root extract of *W. somnifera,* except the control and kept for 24 and 48hrs of incubation. The percentage of wound healing is measured using Image J software and photographed using an inverted microscope.

$$\text{Wound Healing \%} = A_0 - A_T / A_0 \times 100$$

Were; A_0 = initial wound area (24hrs)
A_t = the wound area after 'n' hrs of the initial scratch (48hrs).

Results

Aqueous root extract of *W. somnifera*

Powdered form of *W. somnifera* dissolved in DW and incubated for 18hrs. Later the crude extract was used for further studies.

Fig 3: - Aqueous root extract of *W. somnifera*

Phytochemical analysis of *W. somnifera*

The freshly prepared aqueous root extract of *W. somnifera* were qualitatively analysed for the presence of phytochemical constituents. The test showed the presence of flavonoids and absence of alkaloids, tannins and saponins in the aqueous root extract of *W. somnifera*.

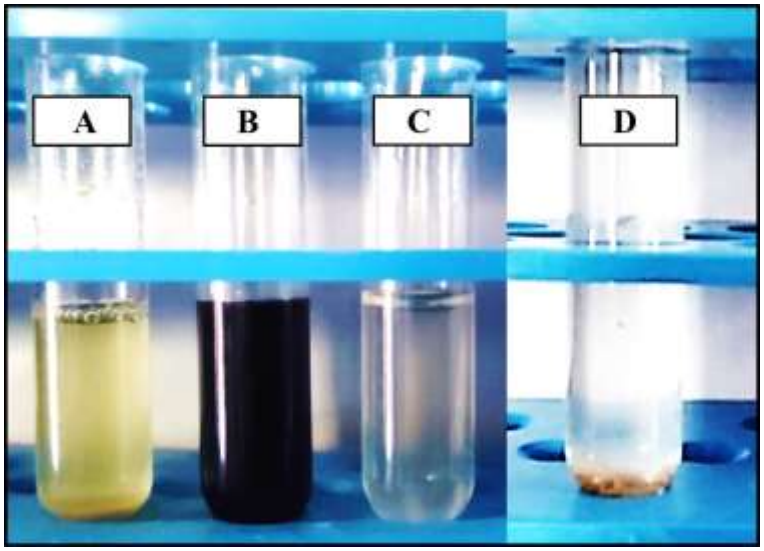


Fig 4: - Screening of Phytochemicals in the aqueous root extract of *W. somnifera* (A - Tannins, B - Alkaloids, C - flavonoids, D - Saponins).

Table 1: Phytochemicals in the aqueous root extract of *W. somnifera*

S.NO	PHYTOCHEMICALS	OBSERVATIONS	INFERENCE
1	Tannins	No blue colour was observed	Absence of tannins
2	Alkaloids	No reddish-brown colour was observed	Absence of alkaloids
3	Flavonoids	A yellow colour was disappeared when acid was added	Presence of flavonoids
4	Saponins	No foam was observed	Absence of saponins

Antioxidant activity of aqueous root extract of *W. somnifera*

The antioxidant activity of aqueous root extract of *W. somnifera* at different concentrations (50µg/ml, 100µg/ml, 150µg/ml, and 200µg/ml) are given below. Ascorbic acid was taken as positive control (PC). OD was measured at 700nm. The rate of absorbance increased with increase in concentration.

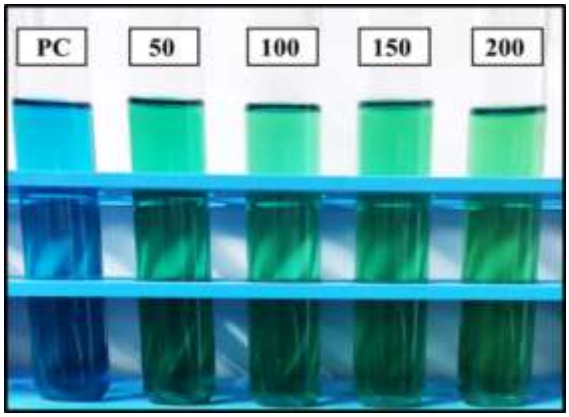
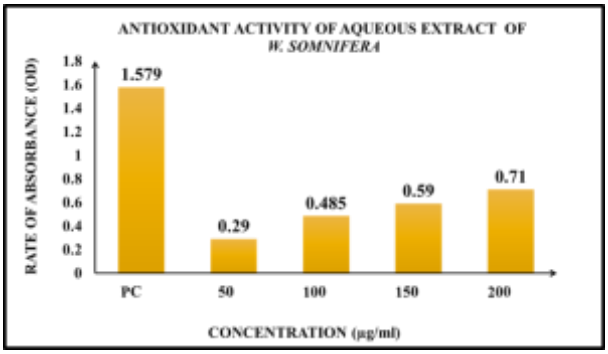


Fig 5: Antioxidant activity of aqueous root extract of *W. somnifera* at different concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml).

Table 2: Rate of absorbance of aqueous root extract of *W. somnifera* at different concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml).

CONCENTRATION (µg/ml)	RATE OF ABSORBANCE (OD)
PC	1.579
50	0.290
100	0.485
150	0.590
200	0.710



Graph 1: Rate of absorbance of aqueous root extract of *W. somnifera* at different concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml).

Anti-cancer activity of aqueous root extract of *W. somnifera*

The anti-cancer activity of aqueous root extract of *W. somnifera* was carried out in MCF-7 cell line using MTT assay and formazan crystals was observed at different concentrations(50µg/ml, 200µg/ml, 300µg/ml & 500µg/ml). OD was read at 570nm. As the concentration increased, the cell viability decreased and % inhibition increased. The higher concentration (500µg/ml) showed minimum cell viability with maximum inhibition.

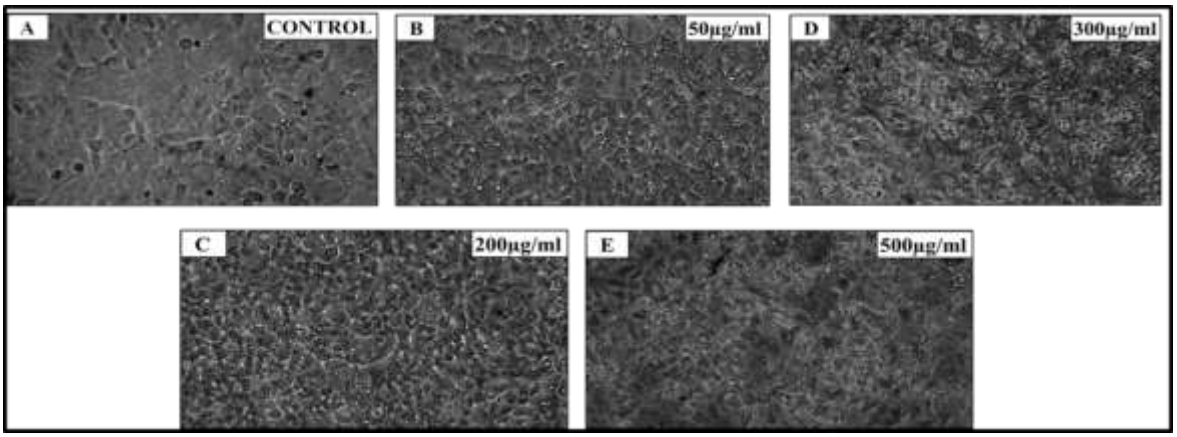


Fig 6: *In-vitro* anticancer activity of aqueous root extract *W. somnifera* (at different concentrations) before addition of MTT on MCF-7 cell line under 20x.

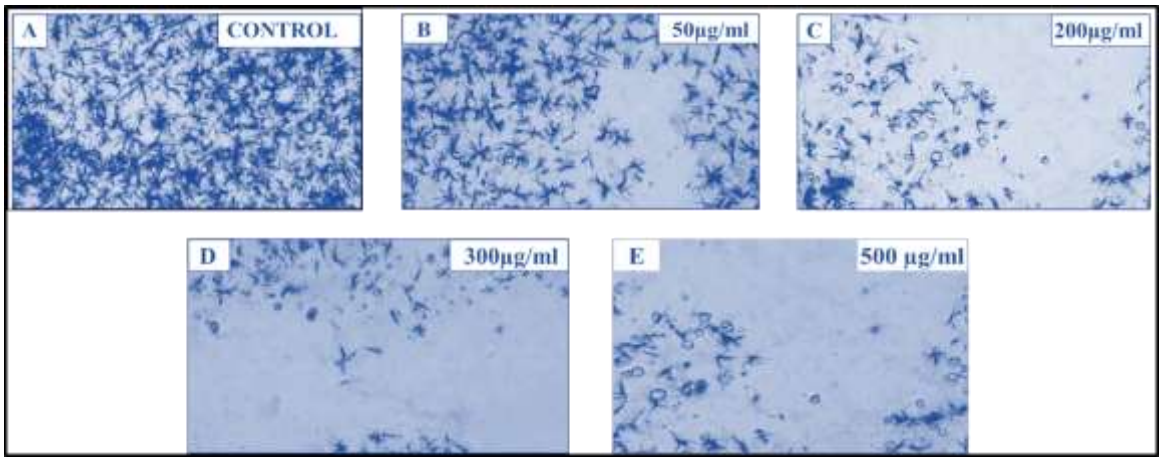
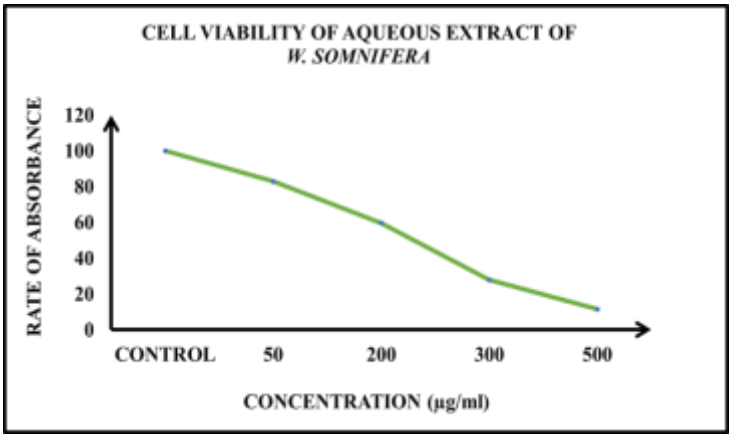


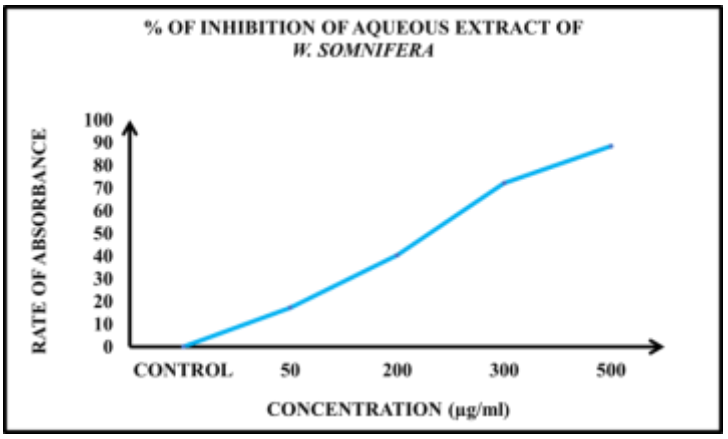
Fig 7: - *In-vitro* anticancer activity of aqueous root extract *W. somnifera* showing the formation of formazan crystals on MCF-7 cell line (after addition of MTT) under 20x at different concentrations.

Table 3: - Rate of absorbance of aqueous root extract of *W. somnifera* at different concentrations on MCF-7 cell line.

S.NO	SAMPLE CONCENTRATION	ABSORBANCE	CELL VIABILITY	% OF INHIBITION
1	CONTROL	0.104	100	0
2	50	86.69	82.89	17.30
3	200	0.062	59.61	40.38
4	300	0.029	27.88	72.11
5	500	0.012	11.53	88.46



Graph 2: Anticancer activity of aqueous root extract of *W. somnifera* showing cell viability at different concentrations (50µg/ml, 200µg/ml, 300µg/ml, 500µg/ml).



Graph 3: Anticancer activity of aqueous root extract of *W. somnifera* showing % Inhibition at different concentrations (50µg/ml, 200µg/ml, 300µg/ml, 500µg/ml).

Antimicrobial activity of aqueous root extract of *W. somnifera*

The antimicrobial activity of different concentrations (50µg/ml, 200µg/ml) of aqueous root extract of *W. somnifera* against *S. aureus*, *P. aeruginosa* and *A. niger* are given below. No antimicrobial activity observed by *W. somnifera* against *S. aureus* and *A. niger*, whereas zone of inhibition observed for aqueous root extract of *W. somnifera* against *P. aeruginosa.*

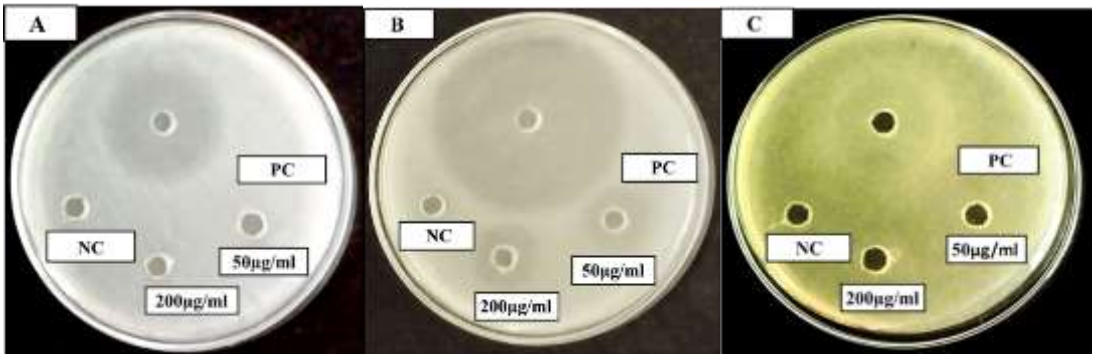


Fig 8: - Antimicrobial activity of aqueous root extract of *W. somnifera* against (A) *S. aureus* (B) *P. aeruginosa &* (C) *A. niger* - 24hrs of incubation at different concentrations (50µg/ml & 200µg/ml).

Table 4: - Zone of inhibition of aqueous root extract of *W. somnifera* against (A) *S. aureus*, (B) *P. aeruginosa &* (C) *A. niger* - 24hrs of incubation at different concentrations (50µg/ml & 200µg/ml).

SAMPLE CONCENTRATION (µg/ml)	ZONE OF INHIBITION – (mm)		
	S. aureus	*P. aeruginosa*	*A. niger*
Gentamicin (PC)	27mm	53mm	26mm
Distilled water (NC)	0mm	0mm	0mm
50	0mm	18mm	0mm
200	0mm	19mm	0mm

Anti-inflammatory activity of aqueous root extract of *W. somnifera*

The anti-inflammatory activity of aqueous root extract of *W. somnifera* at different concentrations (800µg/ml, 1000µg/ml, 1200µg/ml) are given below. Aqueous root extract of *S. cumini* was taken as PC and DW as negative control. OD was measured at 660nm. Thus aqueous root extract of *W. somnifera* showed maximum % inhibition for concentration (1000µg/ml - 91.16).

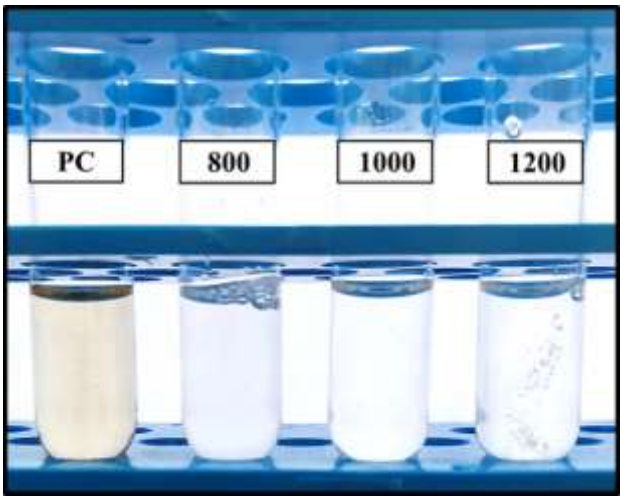
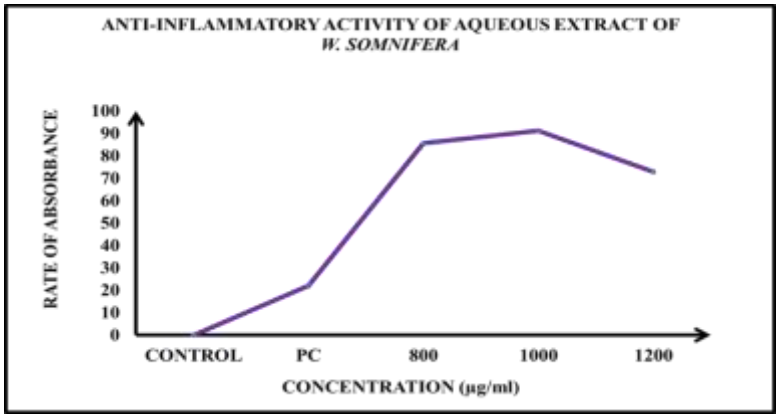


Fig 9: Anti-inflammatory activity of aqueous root extract of *W. somnifera* at different concentrations (800µg/ml, 1000µg/ml, 1200µg/ml).

Table 5: - Rate of absorbance of aqueous root extract of *W. somnifera* at different concentrations (800 µg/ml, 1000µg/ml, 1200µg/ml).

CONCENTRATION (µg/ml)	RATE OF ABSORBANCE (OD)	% OF INHIBITION
CONTROL	0.181	00
PC	0.141	22.09
800	0.026	85.63
1000	0.016	91.16
1200	0.049	72.92



Graph 4: Anti-inflammatory activity of aqueous root extract of *W. somnifera* at different concentrations (800µg/ml, 1000µg/ml, 1200µg/ml).

Anti-diabetic activity of aqueous root extract of *W. somnifera*

The anti-diabetic activity of aqueous root extract of *W. somnifera* at different concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml) are given below. Acarbose was taken as PC. OD was measured at 540nm. The colour of the sample changed to cloudy white and the rate of absorbance decreased with increase in % inhibition.

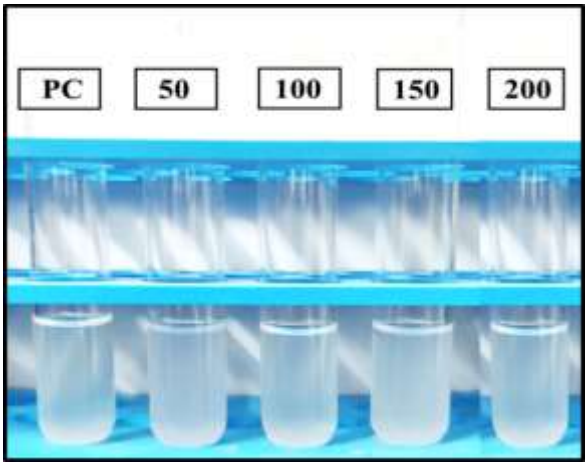
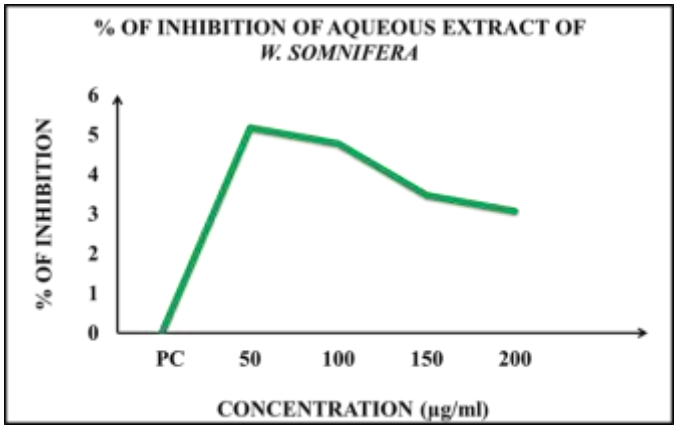


Fig 10: Anti-diabetic activity of aqueous root extract of *W. somnifera* at different concentrations. (PC-Acarbose, 50µg/ml, 100µgml, 150µg/ml, 200µg/ml).

Table 6: Rate of absorbance of aqueous root extract of *W. somnifera* at different concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml).

CONCENTRATION (µg/ml)	RATE OF ABSORBANCE (OD)	% OF INHIBITION
PC	1.467	0.000
50	1.391	5.180
100	1.397	4.771
150	1.416	3.476
200	1.422	3.067



Graph 5: - Anti-diabetic activity of aqueous root extract of *W. somnifera* showing % of inhibition at different concentrations (50µg/ml, 100µg/ml,150µg/ml, 200µg/ml).

Glucose uptake activity of aqueous root extract of *W. somnifera*

The 3T3-L1 cells were treated with glucose and sample (concentration - 200µg/ml). The concentration of glucose uptake was calculated from the standard curve using different concentrations containing glucose (0.002mg/ml, 0.004mg/ml, 0.006mg/ml, 0.008mg/ml, 0.01µg/ml). Aqueous root extract of *W. somnifera* (concentration-200µg/ml) showed more glucose uptake activity than control and PC.

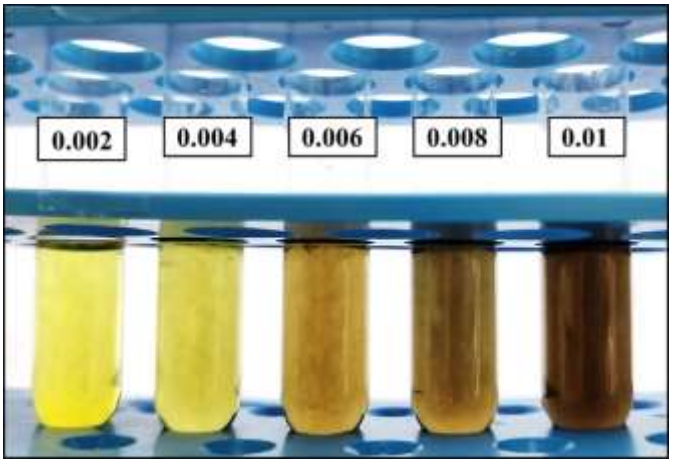
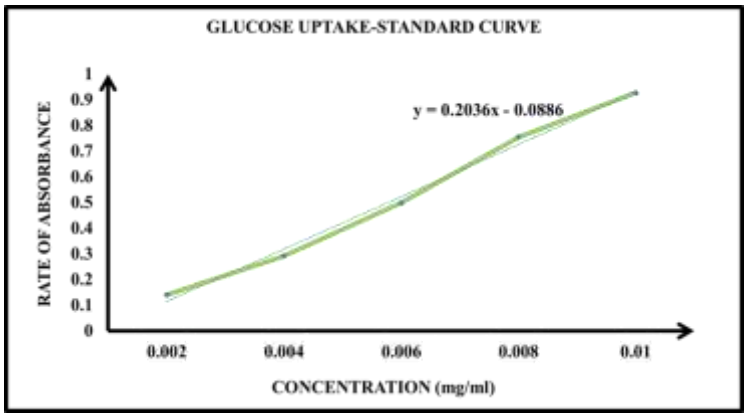


Fig 11: Glucose uptake estimation of standards at different concentrations (0.002mg/ml, 0.004mg/ml, 0.006mg/ml, 0.008µg/ml, 0.01µg/ml).

Table 7: Rate of absorbance of standard glucose at different concentrations (0.002mg/ml, 0.004mg/ml, 0.006mg/ml, 0.008µg/ml, 0.01µg/ml).

S.NO	CONCENTRATION (mg/ml)	ABSORBANCE
1	0.002	0.140
2	0.004	0.292
3	0.006	0.498
4	0.008	0.754
5	0.01	0.927



Graph 6: - Standard curve of glucose uptake at different concentrations (0.002mg/ml, 0.004mg/ml,0.006mg/ml 0.008mg/ml, 0.01mg/ml).

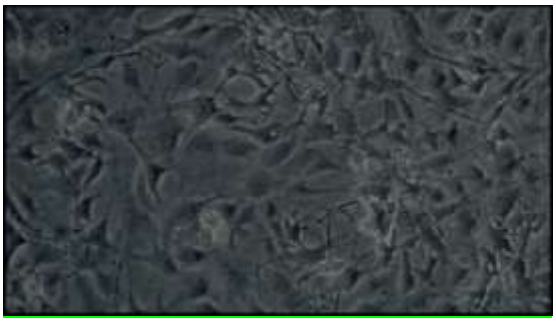


Fig 12: 3T3-L1 cell line in serum free media

Fig 13*: In-vitro* initial glucose uptake activity of aqueous root extract of *W. somnifera* in 3T3-L1 cell line.

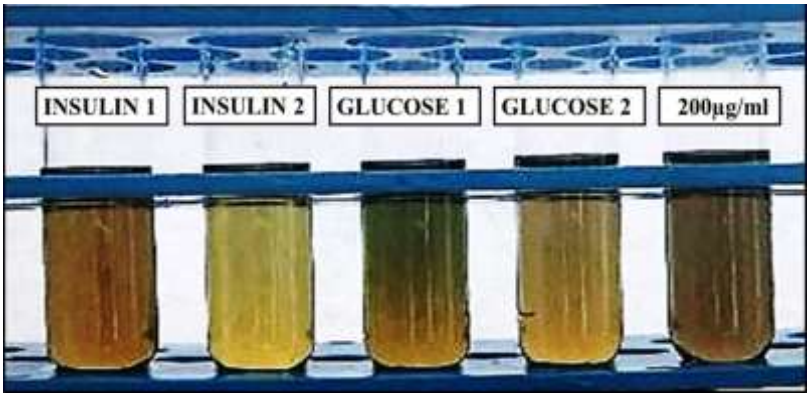


Fig 14: - Initial estimation of glucose uptake showed by aqueous extract of *W. somnifera* (A-B - Insulin+ Glucose, C-D - Glucose, E - Glucose + sample 200µg/ml).

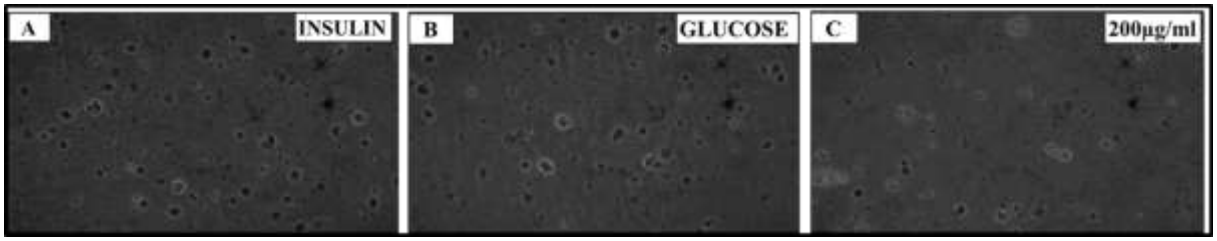


Fig 15: *In-vitro* glucose uptake activity of aqueous root extract of *W. somnifera* in 3T3-L1 cell line after 30mins of treatment with (A - Insulin+ Glucose, B - Glucose, C – Glucose+200µg/ml).

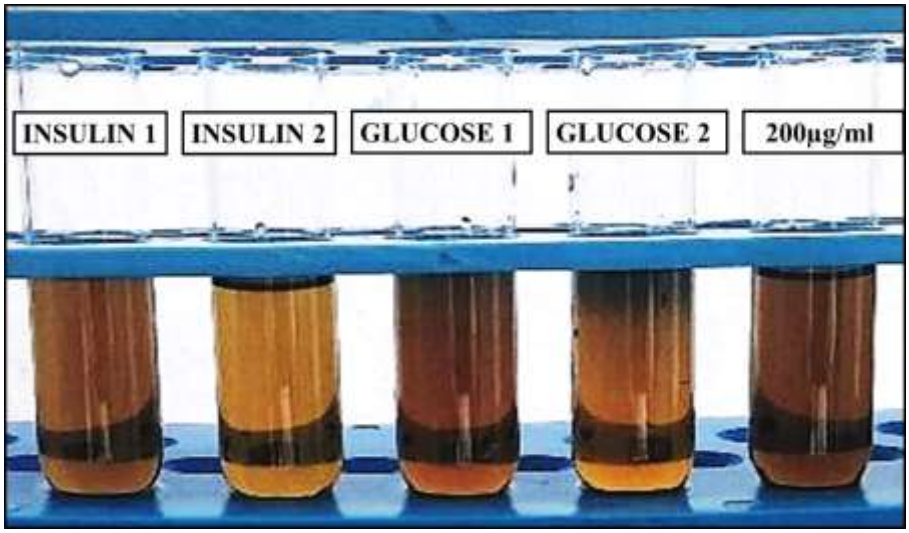


Fig 16: - Final estimation of glucose uptake showed by aqueous root extract of *W. somnifera* after (30mins) incubation. (A-B - Insulin+ Glucose, C-D - Glucose, E - Glucose + sample 200µg/ml).

Table 8: - Rate of glucose uptake activity showed by aqueous root extract of *W. somnifera* at different concentrations in 3T3-L1 cell line

S.No	SAMPLE CONCENTRATION (µg/ml)	INITIAL ABSORBANCE	FINAL ANSORBANCE	INITIAL CONCENTRATION	FINAL CONCENTRATION	% OF GLUCOSE UPTAKE
1	INSULIN 1	0.118	0.106	1.014	0.955	94.18
2	INSULIN 2	0.028	0.013	0.572	0.499	87.23
3	GLUCOSE 1	0.426	0.241	2.527	1.618	64.02
4	GLUCOSE 2	0.334	0.193	2.075	1.383	66.65
5	200	0.397	0.478	2.385	2.782	116.64

Glut - 4 expression in 3T3-L1

The PCR product of cells treated with 200µg/ml concentration of the sample, showed higher expression of GLUT- 4 compared to control (glucose treated cells). GLUT- 4 expression is enhanced by the aqueous root extract of *W. somnifera* as shown in figure given below.

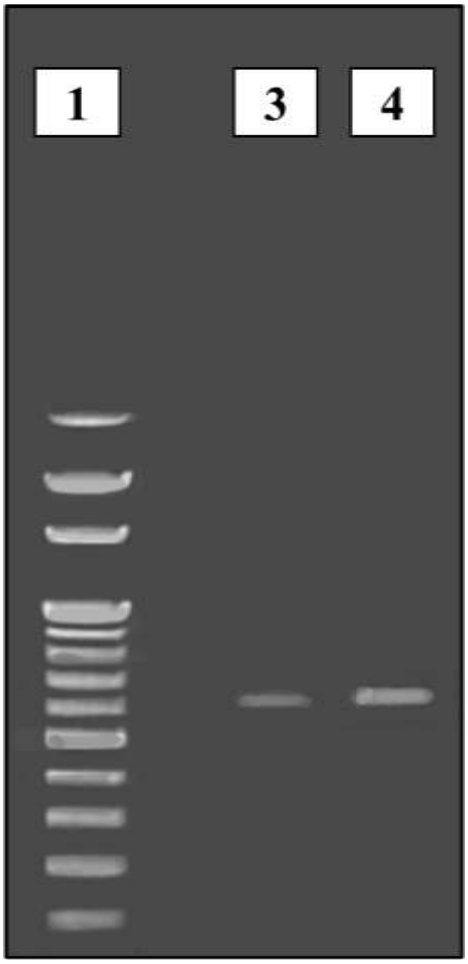


Fig 17: - GLUT- 4 Expression on glucose and sample treated aqueous root extract of 3T3-L1 cell line. (1 – 100 bp ladder, 3 - control, 4 - 200µg/ml).

Wound healing activity of aqueous root extract of *W. somnifera*

The wound healing activity of aqueous root extract of *W. somnifera* at different concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml) in L929 cells are given below. It was observed that the rate of wound healing was rapid in the sample containing the highest concentration of aqueous root extract of *W. somnifera* (200µg/ml) with a wound closure of 25.97%.

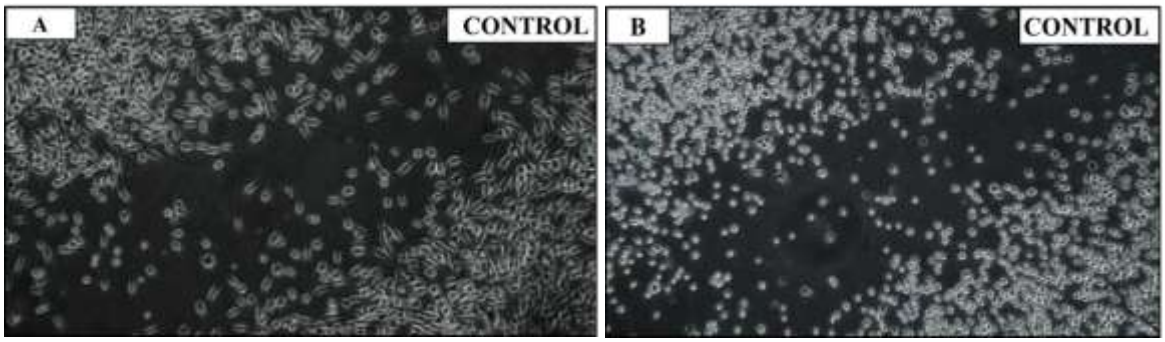


Fig 18: - *In-vitro* wound healing activity on L929 cells taken as control. A - 24hrs & B - 48hrs under 10x.

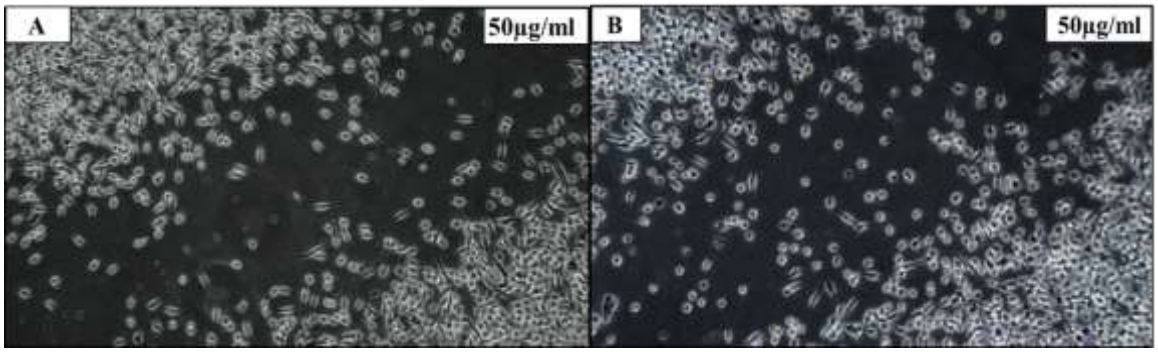


Fig 19: - *In-vitro* wound healing activity of aqueous root extract of *W. somnifera* (50µg/ml) on L929 cells. A - 24 hrs & B - 48hrs under 10x.

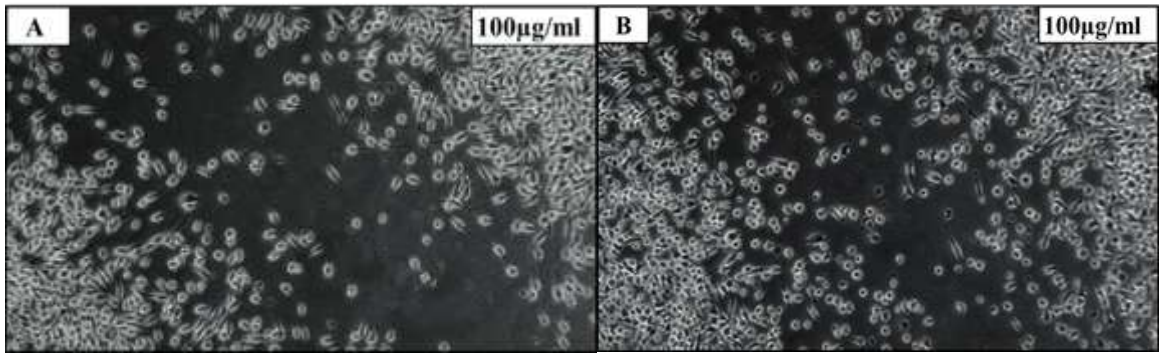


Fig 20: - *In-vitro* wound healing activity of aqueous root extract of *W. somnifera* (100µg/ml) on L929 cells. A - 24 hrs & B - 48hrs under 10x.

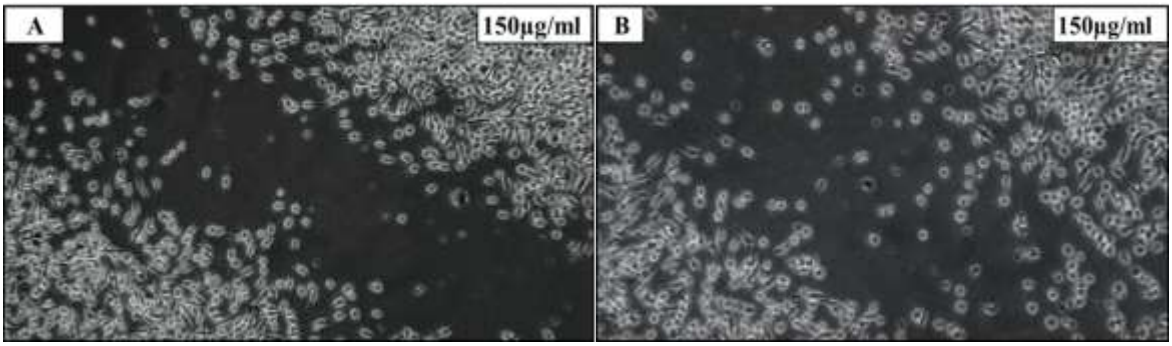


Fig 21: - *In-vitro* wound healing activity of aqueous root extract of *W. somnifera* (150µg/ml) on L929 cells. A - 24hrs & B - 48 hrs under 10x.

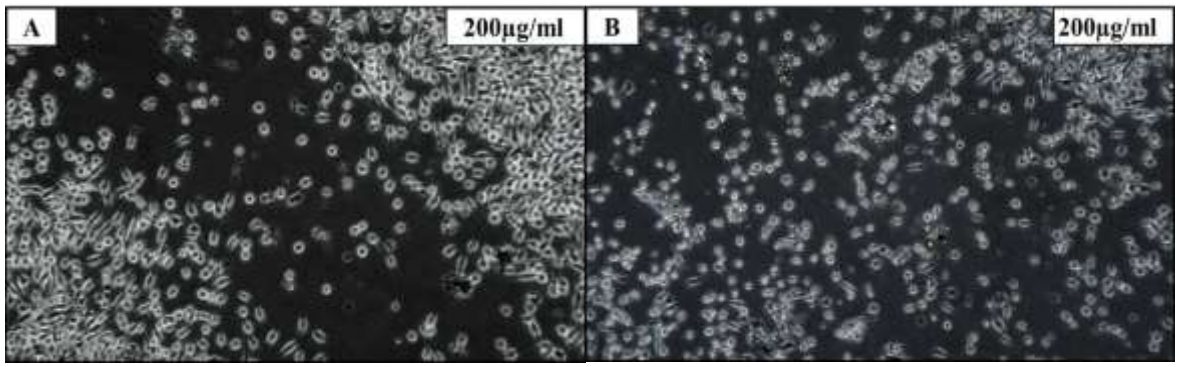
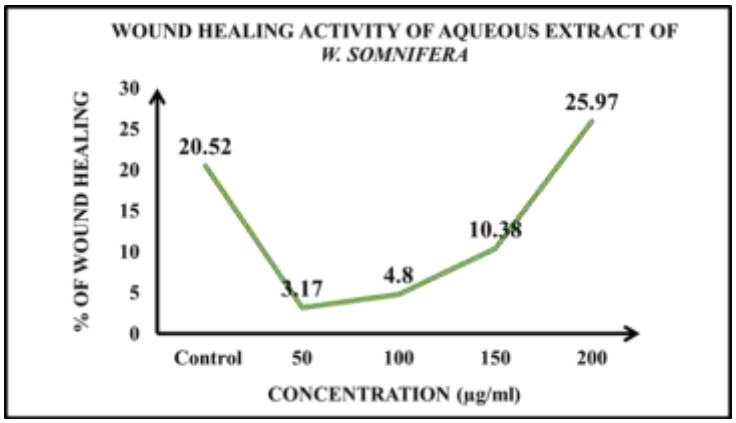


Fig 22: - *In-vitro* wound healing activity of aqueous root extract of *W. somnifera* (200 µg/ml) on L929 cells. A - 24hrs & B - 48 hrs under 10x.

Table 9: - Rate of wound healing activity showed by aqueous root extract of *W. somnifera* at different concentrations in L929 cells.

CONCENTRATION (µg/ml)	% OF WOUND HEALING
CONTROL	20.52
50	3.17
100	4.80
150	10.38
200	25.97



Graph 7: - Wound healing activity of aqueous root extract of *W. somnifera* at different concentrations in L929 cells.

Discussion

In this study, the aqueous root extract of *W. somnifera* was examined to assess its phytochemical composition, antioxidant potential, anticancer, antimicrobial properties, anti-inflammatory effects, anti-diabetic activity, glucose uptake potential and wound healing activities. The phytochemical analysis of aqueous root extract of *W. somnifera* confirmed the presence of flavonoids, which has been previously reported (Ganguly, *et al*., 2018). In our study anticancer activity of aqueous root extract of *W. somnifera* was observed on MCF-7 cell line. (Visweswari, *et al*., 2013) has previously reported that the anticancer activity of aqueous root extract was due to the presence of flavonoids. The antimicrobial activity of aqueous root extract of *W. somnifera* showed effectiveness in inhibiting the growth of *P. aeruginosa* which was reported previously using disc diffusion method by (Barnes, *et al*., 2015), but lacked its activity against *Staphylococcus aureus* and *Aspergillus niger*. The antibacterial activity of aqueous root extract of *W. somnifera* is due to the presence of flavonoids (Singh and Kumar, 2014). Presence of alkaloids and saponins

played a crucial role in antifungal activity (Mishra, *et al*., 2020). Thus, the absence of saponins and alkaloids could be one of the reasons for the lack of antifungal activity in our study. The aqueous root extract of *W. somnifera* showed anti-inflammatory activity. Study showed anti-inflammatory activity of hydro-alcoholic root extract of *W. somnifera* due to antioxidant property (Lalit and Arun, 2014). The glucose uptake potentials expressively stimulate anti-diabetic activity which has been previously studied (Naresh, *et al*., 2018). Presence of flavonoids helps in regulating glucose levels, which could be beneficial in diabetes treatment. (Khalid, *et al*., 2019). Expression of GLUT-4 gene was significantly enhanced by aqueous root extract of *Withania somnifera*. The *in-vivo* study in another species of *Withania* namely *Withania coagulans* showed significant glucose uptake and GLUT-4 expression (Shukla, *et al*., 2022). The ability to promote % of wound closure of aqueous root extract of *W. somnifera* in L929 cells indicated wound healing activity. *In-vivo* wound healing activity of alcoholic extract of *W. somnifera* has been studied previously by (Shawqi, *et al*., 2019) and the action was due to the presence of antioxidant activity.

Conclusion

The experiment was carried out to evaluate the presence of phytochemical, antioxidant, anti-cancer, antimicrobial, anti-inflammatory, anti-diabetic, glucose uptake, wound healing, activity of aqueous root extract of *W. somnifera* and were tested using different methods. From the present investigation it can be concluded that the presence of flavonoids may account for antioxidant activity, anti-cancer, anti-microbial, anti-inflammatory, glucose uptake and wound healing activity of aqueous root extract of *W. somnifera*. Aqueous root extract of *W. somnifera* has great potential to inhibit the growth of *Pseudomonas aeruginosa* but it didn't show activity against *Staphylococcus aureus* and *Aspergillus niger*. *In-vitro* experiments confirmed the ability of aqueous root extract of *W. somnifera* to inhibit diabetes in a dose-dependent manner, indicating its potential as a treatment option for type 2 Diabetes Mellitus. The glucose uptake and wound healing activity was also significant. The glucose uptake enhancement potential was confirmed at molecular level by expression of GLUT-4 gene.

In our study, we investigated and found that aqueous root extract of *W. somnifera* has phytochemical (flavonoids), antioxidant, anti-cancer, antimicrobial, anti-inflammatory, anti-diabetic, glucose uptake and wound healing properties. Further studies can also be undertaken to analyze extracts from different parts of *W. somnifera* to uncover the diverse potential of its natural compounds (steroids, phytophenols, glycosides, etc.), which could contribute new and innovative treatments in Ayurveda.

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